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Introduction to the symposium “Cephalopods: A behavioral perspective”*

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Behavior is not an area we usually associate with molluscs, and one tends to think instead of vertebrates, especially mammals. Yet when we do think of molluscan behavior, it is the cephalopods that come to mind. With their large centralized brain, reputed high intelligence, efficient physiology, and complex motor output, cephalopods have an excellent basis for complex behavior. Despite this capacity, cephalopod behavior is little known and not well explored, and the authors in this symposium, especially the paper collection, attempt to shine light into various corners with a wide variety of cephalopod subjects.

One of the simplest aspects of behavior is sensory reception, and one of the ‘simplest’ systems and most molluscan-general is that found in *Nautilus* Linnaeus, 1758. Soucier and Basil discuss a pioneering laboratory investigation of tactile sensitivity in the nautiloids; clearly these deep-sea animals should rely on non-visual information much of the time, but only their chemical sensing has been well investigated. Now that their mechanical reception has been established, further research will no doubt look more at its use in natural situations and the limits of and receptors for its sensitivity.

The programming of motor output, the root of behavior, is similarly simple on the surface. Grasso has tackled the motor output of suckers of *Octopus* Cuvier, 1797 and their combinations to produce actions on the environment. While movement ought to be simple, the use and coordination of hundreds of suckers turns out to be, as befits the complexity of neural support of the suckers, both complex and variable. How much of this programming is central and how much peripheral as well as how the ‘reflex’ arm control system can perform such complex maneuvers remains to be investigated; again the foundation has been laid for further investigation.

Behavior is linked to the underlying physiology of the animal, and the thoughtful paper by King and Adamo makes sense of the paradoxes in the combination of *Sepia* Linnaeus, 1758 cuttlefish linkage of mantle contraction and blood circulation. The motor action of mantle contraction has a major effect on the circulation of blood through this area, and the authors evaluate why the particular patterns of blood flow during this major event occur. More of such behavior-physiological linkage is needed, and the King and Adamo paper is a welcome start.

One unique, coleoid cephalopod motor system is responsible for the chromatophore system that produces skin patterns and colors, but its complexity means that it is often characterized only informally. Leite and Mather use a computerized data analysis approach to build the repertoire of one *Octopus* species. Such characterization offers insight into the neural production of patterns and pattern complexity on the skin; in addition, this approach may assist us in taxonomic investigation of the species complex of *Octopus vulgaris* Cuvier, 1797.

Behavior gives us insights into physiology and ecology of animals, and behavior of deep-sea octopods in underwater videos is the subject of the paper by Voigt. Because humans are very limited in their activities in the deep sea, cephalopod research has focused on the easily available near-shore and near-surface species of *Octopus*, *Sepia*, and *Loligo* Lamarck, 1798. Thus, Voight’s insight into how these deep-sea and little-known animals behave is particularly welcome.

The most complex areas of behavior are the emergent aspects such as play, personality, and cognition, studied mainly in *Octopus* so far. Mather covers the research in these areas and suggests that we have much to learn about the intelligence, cognitive capacity, and even possible consciousness in cephalopods. She challenges us to look at behavior of molluscs, particularly in but not limited to cephalopods, for greater underlying subtly and complexity than we have assumed so far.

In addition to these published papers, other symposium participants presented work on a range of interesting aspects of cephalopod behavior. Huffard discussed octopus mating strategies for *Abdopus* Norman and Finn, 2001; Cosgrove discussed the brooding behavior of *Enteroctopus* Rochebrune and Mabille, 1889. Again with *Enteroctopus dofleini* (Wülker, 1910) as a model, Lyons and Scheel discussed the ecological impact and movement of octopuses in their natural environment. Finally, Williams looked at the chemical defenses of hatchling *Hapalochlaena* Robson, 1929, and Bush discussed why deep-sea squid might ink into the dark.

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Chambered nautilus (*Nautilus pompilius pompilius*) responds to underwater vibrations*

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Abstract: The deep-water cephalopod *Nautilus pompilius pompilius* Linnaeus, 1758 may benefit from detecting potential signals such as mechanical and acoustical stimuli in its dark habitat where visual information is often limited. Here we examined whether specimens of chambered nautilus are capable of responding to waterborne vibration—a sensory mechanism that has yet to be investigated. We measured the ventilation rate of animals responding to a vibrating bead that produced a range of displacements and velocities. We found that nautiluses do indeed respond to underwater acoustical stimuli, decreasing their ventilation in the presence of a vibratory stimulus. Vibrations resulting from large-bead displacements and high source-velocities caused the animals to decrease their ventilation the most. Stimuli <20 cm from the animals caused a further reduction in their ventilation rates than those at greater distances. These nocturnal animals, living in dark conditions where visual information is often limited, may benefit from including vibrations in the suite of stimuli to which they can respond.

Key words: cephalopods, acoustics, behavior, ventilation, source-displacement

Organisms must cope with a variety of stimuli in the marine environment, and the ability to process this information may contribute to both survival and reproduction. Because the marine environment is dominated by mechanical and acoustical energies, such as water currents or vibrations that may eventually be converted to sound waves, it is a reasonable assumption that many organisms, including *Nautilus pompilius pompilius* Linnaeus, 1758, may benefit from the ability to detect and respond to these varying types of stimuli.

In the last three decades, researchers have identified the variety of sensory systems that contribute to the survival and functional ecology of the chambered nautilus (*e.g.*, Budelmann and Tu 1997). *Nautilus pompilius pompilius* has served as a model in studies of olfaction, vision, and equilibrium reception. Nautiluses, although predominantly chemotactic, are capable of using many sensory systems to complete basic survival tasks (*vision*: Muntz 1991, 1994a, 1994b, *equilibrium reception:* Budelmann 1977, Neumeister and Budelmann 1997, *olfaction*: Basil *et al.* 2000, 2002, 2005). Here we demonstrate that *Nautilus pompilius* is also capable of detecting and responding to underwater vibrational stimuli.

Nautilus pompilius is considered to be one of the oldest members of the class Cephalopoda (phylum Mollusca). Presently, the genus represents less than 1% of the entire cephalopod assemblage (Wood and O'Dor 2000). Nautiluses are the only extant hard-shelled cephalopod, and are therefore commonly used as a modern analog of the ellesmeroceratids, an ancestral lineage that dates back *ca.* 500 Ma (Ward 1987, Wray *et al.* 1995, Ward and Saunders 1997). Nautiluses are bottom dwellers but are not completely restricted to the sediment (nektobenthic). They make daily vertical migrations at dawn and dusk along coral reef slopes throughout the Indo-Pacific, including the Philippines, Palau, Fiji, Papua New Guinea, Australia, Samoa, and Tonga (Ward 1987, O'Dor *et al.* 1993). Nautiluses have limited visual abilities and detect light wavelengths only shorter than 650 nm, with the most efficient absorption occurring at 467 nm (Muntz 1986). They also inhabit a primarily aphotic environment and are commonly found at depths of 150-300 m. Because the internal environment of their shell is resistant to pressure change, nautiluses dwell in depths up to 803 m before shell implosion occurs (Saunders and Landman 1987, Jordan *et al.* 1988).

Nautiluses are slow moving and non-visual, and in general their life history strategies differ greatly from their highly visual relatives, octopuses, squids, and cuttlefish (subclass Coleoidea), which typically live at shallower depths although not exclusively. Aside from life-history strategies,

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nautiloids and coleoids differ in external morphology as well. Coleoids typically possess 8-10 appendages (arms and/or tentacles), all of which are lined with mechanoreceptors and chemoreceptors with the latter occurring particularly within the suckers (Hanlon and Messenger 1996, Cheng and Caldwell 2000, Messenger 2001). Nautiloids have 90-94 tentacles that are typically covered with mechanoreceptor and chemosensory cells (Hamada *et al.* 1978, Fukada 1987, Ruth *et al.* 2002). Nautiloids also have a gas-filled external shell that is sectioned into chambers. Coleoids possess highly developed eyes with lenses that form distinct images. The eyes of *Nautilus* lack a lens but are capable of forming images and capturing light in dark environments, including bioluminescence (Muntz 1994a, 1994b). Given the vast ecological and morphological differences between coleoids and nautiloids, it is a reasonable prediction that each group would use sensory systems, such as vibration detection, differently.

Sources of sound in the ocean include seismic activity, storm events, man-made contributions, and biological activity. For an animal to identify sound as a stimulus, it must extract a signal from the ambient sound environment or, more informally, from background noise (Rogers and Cox 1988). Sound emission can originate from many different sources, but all sound production begins in a similar fashion: a longitudinal, propagating mechanical wave is generated by a change in volume, physical oscillation, or movement. Disturbances from a change in volume that originate from a single pole, such as a pulsating sphere or the inflation of a teleost swim bladder, are referred to as monopole sources. Dipole sources result from a disturbance in the medium in which the volume of the source remains constant but the signal has two points of origin. Typical examples of dipole sources are spheres that vibrate between two points or the sinusoidal movements of a fish moving through the water column (Kalmijn 1988, Coombs 1994).

The acoustic fields created by these sources can be divided into two components: near-field (or local-flow field) and far-field. Stimuli associated with local-flow fields are dominated by particle velocity, displacement, and acceleration, whereas stimuli associated with the far-field can be more accurately measured in scalar quantities such as pressure and density that reflect only the magnitude of the signal. Non-pelagic animals that live in ocean bottoms, coral reefs, intertidal areas, etc., operate primarily in the local-flow field simply because sound waves do not have adequate space to radiate from the source. Pelagic animals frequently operate within both fields and have sensory systems adapted for detection within each field that are dependent on their spatial location at any given time (Bleckmann 1994). An example of the latter would be fishes that possess both lateral-line systems and otoliths, which serve as overlapping sensory systems. The lateral line detects low-frequency stimuli within only a few body lengths of the source, whereas the otolith organs and other components of the inner ear respond to acoustic reception from the outer reaches of the local-flow field well into the far-field (Kalmijn 1988, Braun *et al.* 2002). A similar model could be applied to nautiluses. A plausible mechanism might be that the immediate source (*i.e.*, a group of snapping shrimp) could be detected through mechanoreceptors located on certain tentacles (Ruth *et al.* 2002) while the progression of the wave through the remainder of the near-field into the far-field could be detected by equilibrium receptor organs such as statocysts (Budelmann 1988, Rogers and Cox 1988, Neumeister and Budelmann 1997).

Williamson (1988) tested vibration sensitivity in the northern octopus *Eledone cirrosa* (Lamarck, 1798) and determined that the hair-cell sensitivity within the statocyst of the octopus was three or four orders of magnitude less sensitive than what average fishes can detect. The statocyst of *E. cirrosa* is therefore not considered to be an auditory organ compared to the auditory or far-field detection systems of fishes, although its threshold sensitivities were similar to those of other aquatic invertebrates. More importantly, these results demonstrated that this organ is sensitive to biologically relevant vibrations. Additional studies have suggested that less sensitive vibration thresholds may enhance coleoid survival by lessening the effect of intense acoustic emissions that odontocete predators use to disorient their prey (Moynihan 1985) and that vibration sensitivity need not be confined to the statocyst, indicating that certain mechanoreceptors may be sensitive to vibration as well (Williamson 1988).

It is this line of logic that suggests that *Nautilus* may detect underwater vibration. The statocysts of nautiluses are more primitive than those of coleoids. Perhaps the extreme external morphological differentiation between nautiluses and coleoids has prevented the evolution of such a complex organ due to space or phylogenetic constraints. Additionally, and perhaps more acoustically relevant, there is the gas-filled external shell of the chambered nautilus. Although this shell and its chambers are thought primarily to compensate for buoyancy, principles of underwater acoustics dictate that the shell may also double as a resonating chamber, thereby potentially nullifying the need for the development of a more complex receptor organ.

MATERIALS AND METHODS

Animals

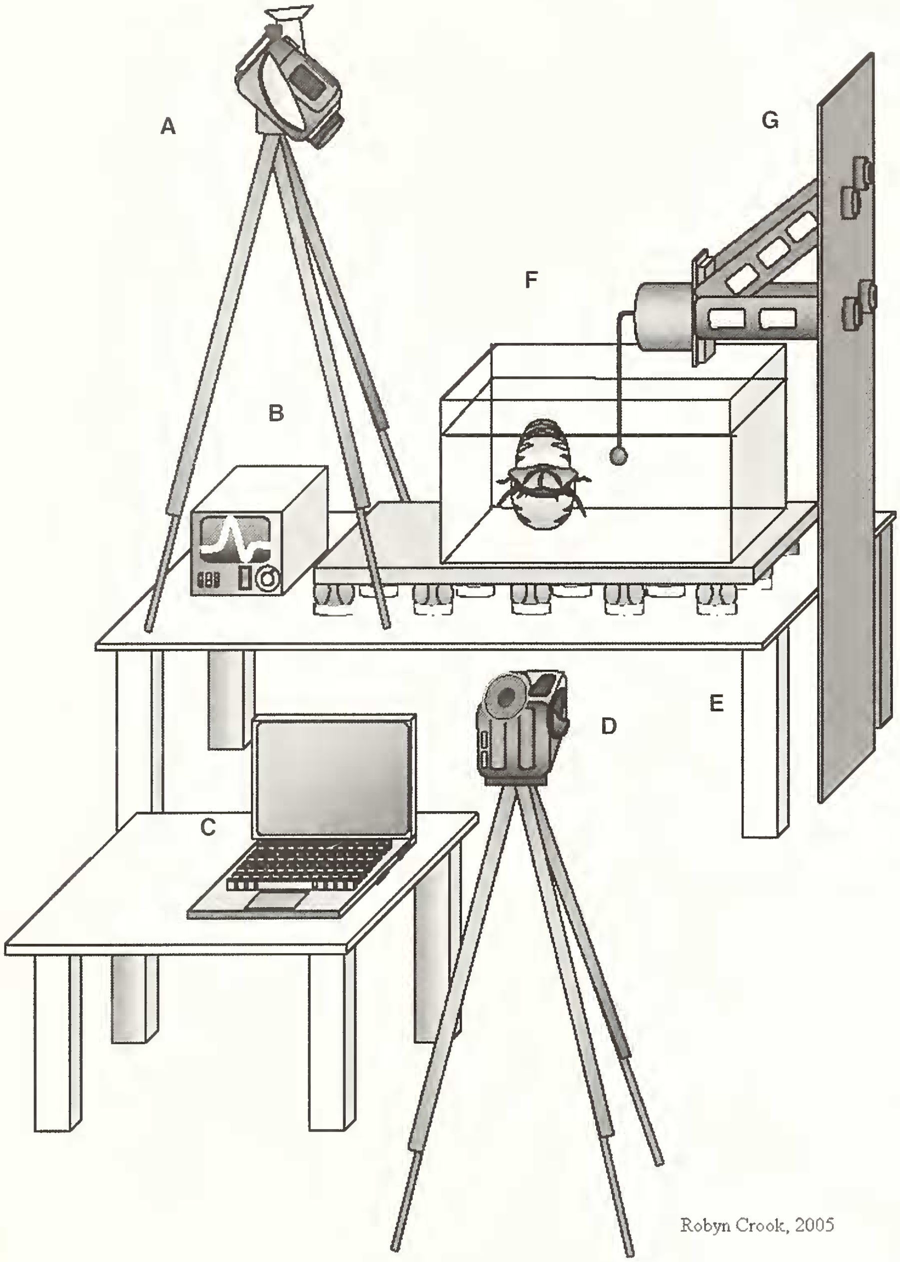
Eleven wild-caught, adult individuals of *Nautilus pompilius*, originally collected in the Philippines and purchased through *Sea-Dwelling Creatures*™, California, were housed in a re-circulating system at the Aquatic Research and Environmental Assessment Center (AREAC) at Brooklyn College of the City University of New York. The animals were divided into two groups and kept separately in a closed system that consisted of two 530-L polyethylene tanks filled with artificial sea water (Instant Ocean™). Both tanks were connected in tandem to a 94.8-L biofilter that contained aeration and filtration media. The animals were kept at constant temperature of 17 °C and at salinities between 32 and 34 psu. Tilapia fish heads (*Oreochromus niloticus eduardianus*) were used as a primary food source, and rations were administered every third day. Daily checks of water quality (temperature, salinity, dissolved oxygen, pH, calcium, alkalinity, ammonia, nitrite, nitrate, and phosphate) were conducted to monitor the system and maintain the health of the animals. Trace elements in the form of a calcium/alkalinity liquid buffer system (B-Ionic™) were added on a weekly basis.

Small and large source-displacement experiments

Experimental apparatus

In two source-displacement experiments (Small Source-Displacement Experiment [SSDE] and Large Source-Displacement Experiment [LSDE]), the experimental arena was a rectangular Plexiglas™ tank (51 cm long × 25.4 cm wide × 31.7 cm tall), containing ~30 cm standing water (Fig. 1). To control for ambient background noise, an insulated and isolated basement room was selected to run the trials. Within the room, the tank was placed on a vibration-absorption table constructed from a granite slab (151 cm × 56 cm × 3 cm). The slab was placed on 12 tennis balls that were separately set in plastic rings and spaced evenly across a metal desk (73.5 cm × 77 cm × 115 cm).

Two digital cameras (Sony Digital Handycam, model DCR-VX1000) mounted on tripods recorded each trial and provided both top and side views. One camera was positioned 1.5 m in front of the long-axis of the tank and the other was placed 1 m above the tank. Visual contact between animals and observers and inadvertent cuing was prevented by placing a removable blind along three sides of the tank and maintaining a minimal distance of 3 m from the uncovered portion of the tank. One fluorescent light bulb was used overhead to illuminate the apparatus, and experimenters did not move in front of the apparatus during the trials.



Robyn Crook, 2005

Figure 1. Experimental setup for source-displacement experiments. A, top-view camera; B, oscilloscope; C, laptop computer; D, side-view camera; E, vibration absorption table; F, experimental tank with animal; G, wall mount with mini-shaker and shaft/bead.

Vibrating stimulus

A dipole source was created by mounting a spherical acrylic bead (18.95 mm in SSDE and 9.44 mm in LSDE) to an aluminum shaft (17 cm in length and 2 mm in diameter) that was bent at a 90° angle and attached to a mini-shaker (Bruël and Kjaer vibration exciter, model 4810). The mini-shaker was fixed to a wall-mounted frame and positioned inside of the tank, such that the bead was located in the middle. Pulse trains were delivered using a laptop computer, and signal outputs were monitored with an oscilloscope (Tenma, model 72-320). Displacement values were based on existing literature (Williamson 1988, Klages *et al.* 2002) and divided into two overlapping ranges that were presented in separate experiments. This format was chosen to minimize habituation to the stimulus and to prevent stress resulting from extended trial times necessary to present the entire range of displacements. The smaller values were tested in the SSDE and ranged from 0.01 to 0.13 mm, whereas the larger values were tested in the LSDE and ranged from 0.08 to 1.12 mm. For the Large Source-Displacement Experiment, a stereo receiver (Kenwood, model VR-615) was used to amplify the signal, thereby increasing the source displacement.

Stimulus signals were created using SigGenRP v.4.4 stimulus design software from Tucker-Davis Technologies. Stimulus presentations were compiled and edited using CoolEdit Pro v.2.1 from Syntrillium Software Corporation recently renamed Adobe Audition v.1.5. Each of the stimulus pulse trains was 5 s long and included ten 2-ms clicks of the same amplitude, separated by nine 0.553-s intervals of silence. Clicks are defined as short, intense bursts of energy that encompass a wide range of frequencies. Stimulus pulses and their respective source-displacements were measured and calibrated prior to the experiment using a Metrolight laser micrometer (model Alpha XO3). All pulse trains were presented only once in each of the trial sequences. Their presentation orders were determined using a random number generator.

Experimental procedures

Trials were conducted on separate days between the hours of 1100 and 1800. The experimental tank was filled with conditioned seawater from the home tank to ensure that each animal was constantly exposed to uniform and familiar olfactory cues. Seven animals were used in the SSDE and five animals were used in the LSDE, three of which were the same (repeated-measures within-subject design; Myers and Well 2003). Animals were transported from the home tank in covered buckets, gently transferred to the test arena, and allowed to habituate for 10 min prior to the start of experimental trials. Following habituation, video recording commenced and individuals were subjected to a 5-min control period during which time no vibrational pulses were administered. The control period was followed by a 5-min "stimulus package" that began with 20 s of baseline silence and continued with the presentation of 11 randomly ordered pulse trains that were separated by 20 s of silence.

Treatment order (control first, stimulus second) was not altered between trials because it was unclear how long the effect of the stimulus on the behavior of the animals, if any, would last. If the stimuli were to be presented before the control in these initial experiments, any continuing effect on the behavior of the animals would reduce the legitimacy of the control data. After trial completion, video recording was stopped and animals were returned to their home tank. The test aquarium was rinsed thoroughly between trials with fresh water to remove any residual individual olfactory cues.

Frequency-sensitivity experiment

Experimental apparatus

The experimental arena was similar to that of the SSDE and LSDE with the exception that a smaller, rectangular Plexiglas™ tank (41 cm × 21 cm × 26.8 cm) containing ~25 cm standing water was used. Additionally, four foam pads that measured 14.5 cm in height were used to absorb background vibration, and only one camera, placed 1.5 m in front of the long axis of the tank, was used.

Vibrating stimulus

Stimulus frequencies were generated in an identical fashion to that described previously in the SSDE section. Stimulus presentations were compiled and edited using CoolEdit Pro v.2.1 from Syntrillium Software Corporation (Adobe Audition v.1.5). The 5-min stimulus package consisted of 11 randomly ordered frequencies (10, 50, 75, 100, 150, 200, 300, 400, 500, 750, and 1000 Hz) that were chosen based on existing literature and by determining which frequencies might be most prevalent in the animal's natural habitat (Williamson 1988, Klages *et al.* 2002). A 0.37 mm bead displacement was used for all frequencies so corresponding source-velocities could later be determined. This value was chosen based on results from the LSDE that revealed that this displacement value caused a large decrease in nautilus ventilation rate and was large enough to eliminate concerns of background interference. Each frequency emission was 5 s long and was separated by 20 s of silence. A selected frequency was included only once per trial sequence and the presentation orders of the frequencies were determined with a random number generator.

Experimental procedures

See *Experimental procedures* from the previous experiment for habituation procedures. Eight animals were used in the frequency-sensitivity experiment (FSE), and trials consisted of a 5-min control period (silence) and a 5-min stimulus-set presentation consisting of 11 randomly ordered frequencies. The presentation of the treatment category (control or stimulus) was alternated between trials, and a 5-min buffer period (silence) was inserted between treatments to control for order effects.

Data collection and behavioral analysis

Data were collected from the video recordings by two independent "blind" observers using a Sony DHR-1000 digital video-cassette recorder. A suite of five typical *Nautilus* behaviors (Basil *et al.* 2005) was identified prior to the experiment but no *a priori* assumptions were made about whether those behaviors would be evident or about their magnitude and polarity. Trials were subdivided into 5-s bins and individual behavioral measurements were recorded in real time for each bin. Typical behaviors such as rocking, touching the bottom of the tank (not just resting on the bottom), tentacle extension (expressed as a percentage of body length), and the "cat's whiskers" foraging posture were not detected in any of the trials. Ventilation rate was a consistent and robust measure of response and has been used as an experimental measure for other cephalopods (King and Adamo 2006) and, hence, will be the focus of all our analyses.

Ventilation rate was defined as the number of completed respirations per 5-s interval and is abbreviated as ventilation rate/5s or VR. This behavior was recorded by observing the area of the mantle cavity bilaterally located posterior to the eye or by minor vertical oscillations of the entire animal produced by water expulsion through the hyponome (Fig. 2). A completed respiration was defined as either (1) the period between one closure of the mantle to the next or (2) the deviation in movement of the animal from a standing position to a position either slightly above or below, and then the return to the initial standing position, which has proven to be another reliable indicator of ventilation in these animals (Basil *et al.* 2005).

Statistical analysis

A repeated-measures within-subject design was used for all three experiments (Myers and Well 2003). Paired samples Student's *t*-tests were used to compare ventilation rates of animals between treatments to determine if exposure to a vibratory stimulus had any effect on behavior. Both control and stimulus periods were 5-min long and data were collected in 5-s intervals or time bins. Data for each time bin were combined and averaged for each treatment and for each animal.

Additional analyses were then performed on data that were divided into categories based on the spatial and temporal response of the animals. Two "distance" categories were created: responses of animals <20 cm and >20 cm from the source. Spearman's Rank correlation tests were used to examine the correlation between distance from the source and ventilatory behavior. In instances where the same animal was used in more than one experiment, a single mean ventilation rate was used to prevent pseudoreplication. This was not possible for analyses that examined potential effects of distance from the source on ventilation rate, as animals that participated in more than one experiment often occupied both distance categories, therefore requiring that the trial averages be separated for analysis.

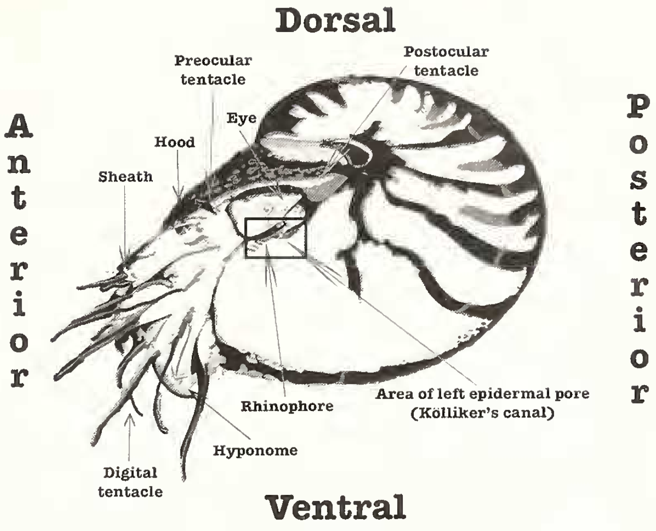


Figure 2. Lateral view of *Nautilus pompilius pompilius*, depicting various external components with emphasis on the location (near the rhinophore and the epidermal pore that connects Kölliker's canal with the left statocyst) of the mantle cavity that was used to count ventilation rates.

To describe the reaction of the animals through time, five temporal categories were created by subdividing the stimulus category. During each trial, a maximum of 11 data points were collected for each of the following stimulus categories: 5-s stimulus presentation (5 s stim), 1-5-s post-stimulus (1-5 s post), 6-10-s post-stimulus (6-10 s post), 11-15-s post-stimulus (11-15 s post), and 16-20-s post-stimulus (16-20 s post). Categorical averages for each trial, and subsequently each animal, were obtained and paired-samples *t*-tests were used to compare control data to each of the 5-s post-stimulus categories.

As an additional note, mean ventilation rates varied greatly between animals so numerical ventilation rates were converted into percentage change from the control to demonstrate changes in behavior graphically. However, all statistical tests were performed on the actual ventilation values as opposed to the percentage values to avoid an artificial increase or decrease in probability due to the imposition of fixed limits (0-100) on the measure.

RESULTS

Overall combined results for all experiments

Twenty trials using 11 animals were conducted. A significant decrease of 8.23% in ventilation rate/5 s occurred between control and stimulus treatments across all animals (Paired-Samples Student's t-test, $N = 11$, $t = 2.61$, $P = 0.03$) with a mean control VR of 4.06, $SD = 1.72$ and a mean VR in the presence of a stimulus of 3.70, $SD = 1.45$.

Mean ventilation rates for *Nautilus* remained below control values for at least 20 s post-stimulus presentation (Fig. 3). Paired-Samples t-tests revealed that the largest decrease of 9.9% was observed during the actual 5-s stimulus presentation (t-test, $N = 11$, $t = 2.90$, $P = 0.02$) and the smallest decrease of 6.9% occurred 5 seconds after that (t-test, $N = 11$, $t = 2.37$, $P = 0.04$). The responses of animals in the remaining three 5-s post-stimulus bins were 8.6% lower than controls in the 6-10 s post-stimulus bin (t-test, $N = 11$, $t = 2.80$, $P = 0.02$), 7.4% lower during the 11-15 s post-stimulus bin (t-test, $N = 11$, $t = 2.26$, $P = 0.048$), and lastly 8.2% lower than controls during the 16-20 s post-stimulus bin (t-test, $N = 11$, $t = 2.26$, $P = 0.05$), respectively.

Data from 15 trials using eight stationary animals were examined to determine if ventilation rate decreases in *Nautilus* when animals are closer to a vibrating stimulus. Only animals that remained stationary throughout the trial were used so their distance from the source would be constant. Five of the animals participated in more than one trial and, unless an animal produced values for both distance categories, their mean VR values were averaged between trials and used in the analysis. Six animals <20 cm from the source had an average of VR 2.83, $SD = 1.07$ whereas six animals that were >20 cm demonstrated a slightly higher average VR of 2.87, $SD = 0.38$. No significant correlation between distance from the source and VR was found (Spearman's Rank correlation, $N = 8$, $r_s = 0.22$, $P = 0.60$). Additionally, a subset of animals was selected for which data existed in both distance categories for each animal. Means from both categories were compared to determine if distance from the source caused significant differences in VR. Although no significant differences were evident (Paired-Samples t-test, $N = 4$, $t = -2.52$, $P = 0.09$), animals vented at a rate that was 8.0% lower when they were closer to the stimulus than when they were >20 cm from the origin of the vibrations.

When source-displacement increased, animals exhibited a decrease in their ventilation. Pearson correlations examined ventilation rates in seven animals from the SSDE and LSDE (Fig. 4) across nine trials. Three animals were <20 cm from the source and six animals were >20 cm from the source. A significant inverse correlation was found between source-displacement and VR for animals that were <20 cm from the source (Pearson correlation, $N = 6$, $r = -0.57$, $P = 0.01$). No significant correlation was found between source-displacement and VR for animals that were >20 cm from the source (Pearson correlation, $N = 3$, $r = 0.43$, $P = 0.06$). On average, animals from the SSDE and LSDE, when exposed to a vibratory stimulus, ventilated at a rate that was 11.72% less than the control VR when they were <20 cm from the source, compared to a 5.38% decrease for those that were >20 cm from the source.

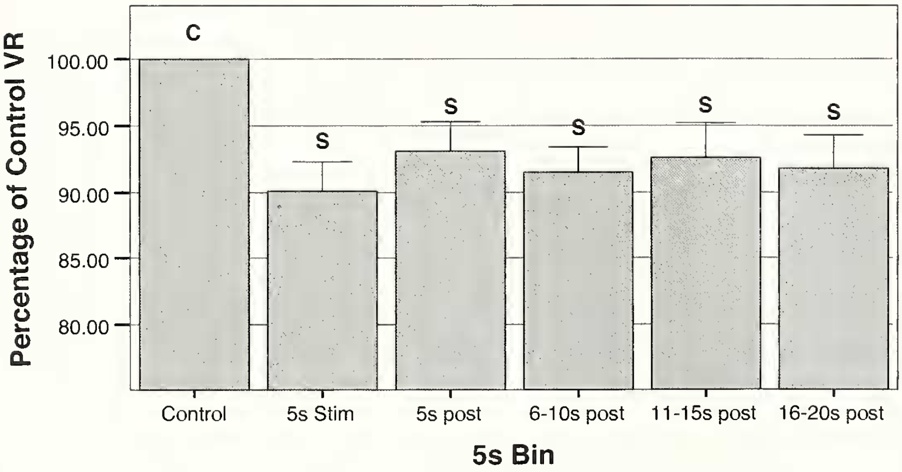


Figure 3. Bar graph depicts the mean percent change in ventilation rate (VR) of the control when compared to each of the five stimulus and post-stimulus time categories. Each bar labeled "S" represents a 5-s period of time that begins with the presentation of the stimuli and continues for a 20-s post-stimuli period. The bar labeled "C" represents a 5-min control period. Significant decreases between the control and stimuli bins were found for each of the five time categories but no continual decrease in VR over time was observed. Error bars show +1 *SE*.

When source-velocity increased, as seen during the FSE, animals also exhibited a decrease in their ventilation. Mean ventilation rates for five animals which were used in the FSE were examined across 12 source-velocity categories (Fig. 5). Four animals were <20 cm from the source and the remaining animal maintained a distance >20 cm from the source. No significant relationship was found to exist between source-velocity and VR

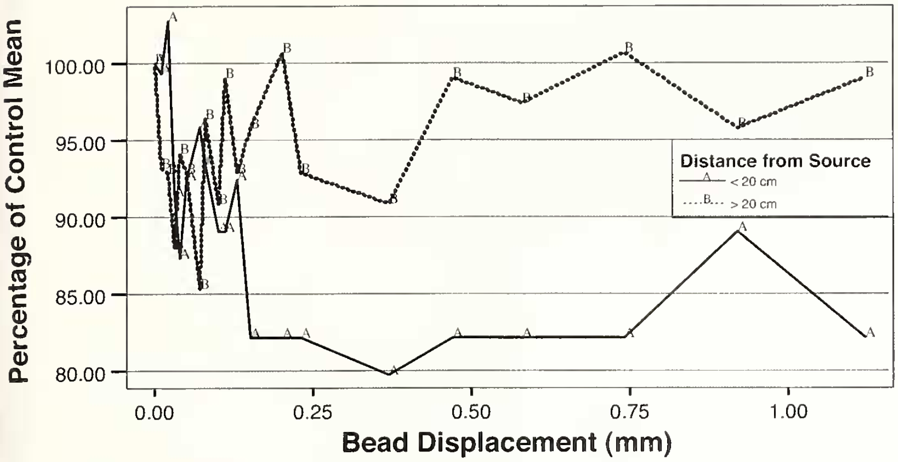


Figure 4. The impact that animal distance from the source and source-displacement has on ventilation rate. Data shown are from two experiments, the Small Source-Displacement Experiment (SSDE) and the Large Source-Displacement Experiment (LSDE), and account for eight animals across nine trials. Three animals were <20 cm and six animals were >20 cm. Bead displacement refers to the distance traveled by the leading edge of the bead and does not include bead diameter.

for animals that were <20 cm from the source (Pearson correlation, $N = 4$, $r = -0.52$, $P = 0.08$). No statistical correlation could be conducted between source-displacement and VR for animals that were >20 cm from the source because of an inadequate sample size ($N = 1$). Animals from the FSE, when exposed to a vibratory stimulus, ventilated at a rate that was 16.3% less than the control VR when they were <20 cm from the source compared to a 0.6% increase for those that were >20 cm from the source.

Additionally, treatment-order effects and the possibility of habituation across trials in nautiluses were examined. The analysis of presentation order, control first or stimulus first, revealed that no treatment-order effect was evident in the FSE (Independent Samples *t*-test, $N = 8$, $t = 1.55$, $P = 1.44$).

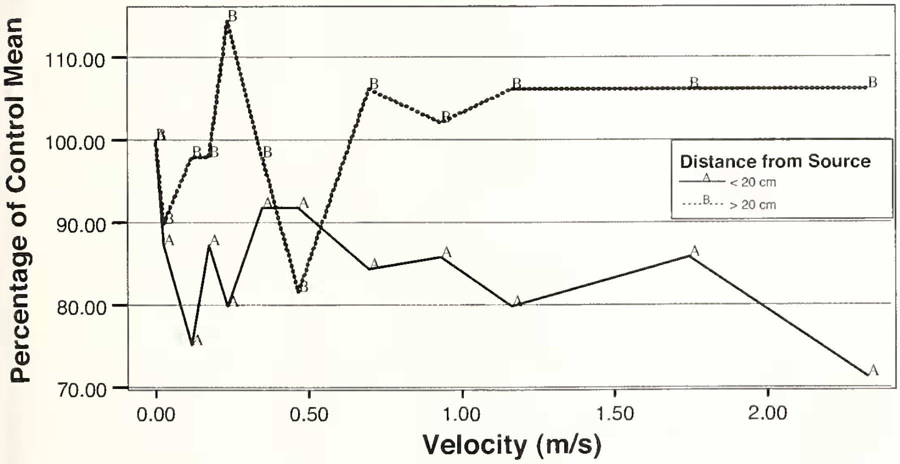


Figure 5. The impact that animal distance from the source and source velocity have on ventilation rate. Data shown are from the Frequency-Sensitivity Experiment (FSE) and account for five animals across five trials. Four animals were <20 cm from the source and one animal was >20 cm from the source. Velocity represents varying source-intensities that were presented randomly.

DISCUSSION

The major finding revealed by these experiments is that *Nautilus* responds to underwater vibrations. Animals almost always reduce their ventilation rate in the presence of a vibratory stimulus: there were significant decreases in ventilation rate during a majority of trials when the animal was exposed to vibratory stimuli.

Comparatively speaking, these findings are relevant to research conducted previously on other invertebrates, such as Williamson's (1988) investigation into the vibrational sensitivity of the statocyst in the northern octopus where a minimum particle-displacement threshold of 0.12 μm was determined and the study conducted by Klages *et al.* (2002) that noted that the deep-water amphipod *Eurythenes gryllus* produced particle displacements of 0.05-0.3 μm between 70 and 200 Hz when feeding and swimming. This work has demonstrated that nautiluses are capable of responding well within these ranges of displacements and frequencies, so future work should focus on determining practical applications of this system in the wild. The detection of signals in the wild can benefit *Nautilus* in many ways. A decrease in ventilation rate could possibly serve as a mechanism for predator avoidance. Similar responses have been observed across multiple groups of animals including cephalopods. King and Adamo (2006) demonstrated that the cuttlefish *Sepia officinalis* Linnaeus, 1758 reduced ventilation and cardiac rates when exposed to sudden visual stimuli, in preparation for a flight response. Additionally, the authors identified four hypotheses in the literature that offered explanations for this behavior, one of which was that animals decrease ventilation to increase crypsis. Although they rejected this hypothesis, suggesting that cuttlefish decrease VR in preparation of a flight response, the hypothesis can be applied to nautiloids since no movements associated with the stimulus were observed during experiments.

From a biological standpoint, decreasing respiratory rates may serve as a defense mechanism. Presumably, approaching predators emit a range of vibratory stimuli resulting from motion, such as the sinusoidal movements of fish. Therefore, such a mechanism would work most effectively in concert with cryptic coloration, by reducing overall rocking movement as the predator nears.

Conversely, decreasing respiration may benefit an animal's predatory success. This is not to imply that nautiluses are formidable hunters—but a sit-and-wait strategy is possible. These animals spend most of their lives associated with coral reefs that are teeming with potential prey items. Perhaps nautiluses, upon detection of certain chemical or vibrational cues, decrease respiration to make themselves less conspicuous to an unsuspecting prey. However, it is improbable that a decrease in VR is an offensive strategy since anecdotal evidence suggests that captive animals increase respiratory activity when exposed to food sources (Soucier, pers. obs.).

Nautiluses likely detect vibration with epithelial tactile receptors on the tentacles, mechanoreceptors below the rhinophore, or some other innervated system. In cuttlefish (Komak *et al.* 2005), epidermal lines along the mantle and arms containing polarized hairs are able to detect local water movements and subsequently integrate that information into behavioral responses. The locations of these potential receptors in *Nautilus* were, however, not ascertained in our experiments. Additionally, the role of the gas-filled external shell acting as a resonating mechanism was not investigated during our experiments but should not be excluded from consideration as a contributing factor.

Irrespective of the mechanism, any additional sensory system that an animal can use, whether it is in conjunction with alternate systems or serving as a primary system would be beneficial to the survival of that animal. Based on the average depth in which these animals live, the nektobenthic niche that they occupy, and the lack of information regarding their feeding and mating strategies, an evolutionary argument could be made for possessing a mechano-sensory system capable of detecting hydrodynamic disturbances and/or substrate-borne vibrations.

In regard to latency of response or time-specific responses, our experiments revealed no temporal trends within our time periods because significant decreases in ventilation rate ranged from the stimulus presentation to the 16-20s post-stimulus period. These animals can respond to the stimulus for up to at least 20 s post-presentation, and the distance from the source and the components of the signal should be the focus of future investigations.

The results of these experiments clearly indicate that *Nautilus pompilius pompilius* can detect and respond to vibrational stimuli. To what end this sensory system serves, whether it is mate selection, prey acquisition, predator avoidance, or a combination of multiple evolutionary functions, has yet to be determined. What has been established is that the recognition of these signals and subsequent behavioral response may pose some type of evolutionary advantage.

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